STUDIES IN TRYPTOPHANE FEEDING I.

by

C. S. HICKS

(From the Biochemical Laboratories, Cambridge, England).

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The Kendall formula for thyroxin, and the more recent work of Wier (1) on the tryptophane content of thyroid gland, suggested that the amino-acid might be the parent substance of the essential secretion. Animal experiments were devised at the instigation of Sir F. G. Hopkins to determine:

(*a*) What changes, if any, occur in the thyroid tissues of an animal on a diet deficient in tryptophane.

(*b*) What changes might similarly occur in heat output.

The first diet chosen was the original Hopkins acid hydrolyzed casein mixture (2).

[1]Amino acid from acid hydrolysis of casein	= 23%
Starch	= 40%
Sugar	= 17%
Fat	= 15%
Salt	= 5%
Cystine	= 1%

[1]Tested for absence of tryptophane.

The animals, which were of uniform stock from Mottram and Hartwell's breeders (3), were divided into the following groups:

(1) Six controls on corn, bread and milk, and vegetables.

(2) Six controls on synthetic diet + tryptophane in amount equal to 2% of total protein fraction.

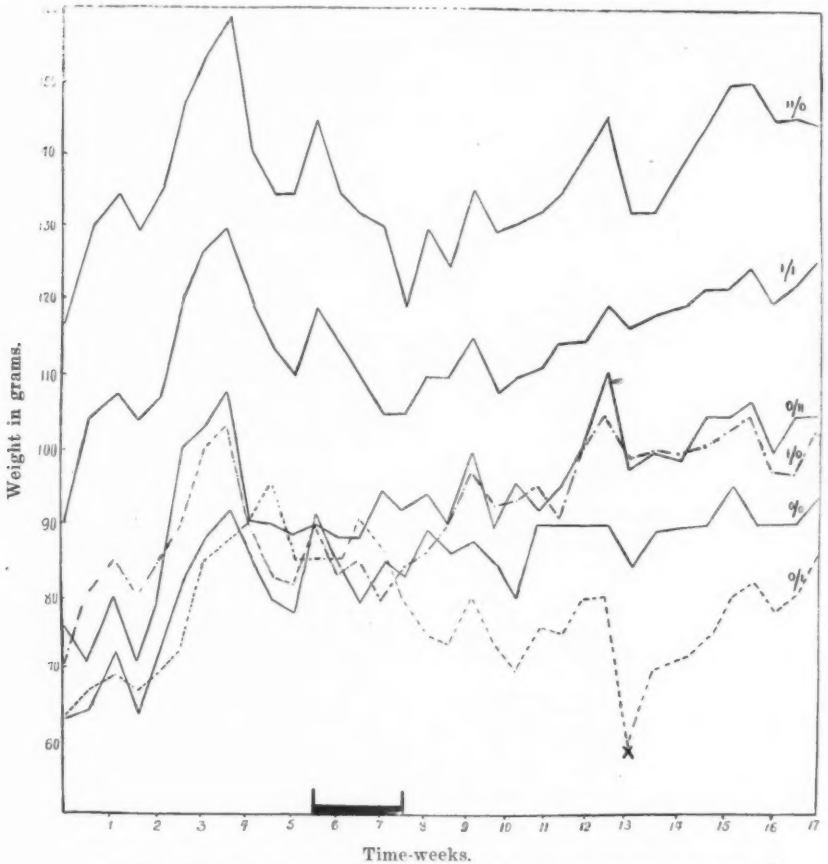
(3) Four rats on synthetic diet containing 0·5% tryptophane.

(4) Six rats on synthetic diet free from tryptophane.

As controls on the specific nature of the deficiency, two rats from Experiment 4 were removed midway through the experimental period and placed on diet (3). Vitamins were supplied in clarified butter, alcohol-cleared marmite extract, and filtered orange juice.

The tryptophane was extracted after preparation by Dakin's butyl-alcohol method, and was free from traces of heavy metals; 80 grms. were prepared.

The animals were watched very closely, and were housed in roomy cages, each 2 ft. 6 in. x 2 ft. x 2 ft. in dimensions, and fitted with exercising wheels. The rats were entirely free from vermin, catarrh, or skin disease, and scrupulous hygiene was maintained.

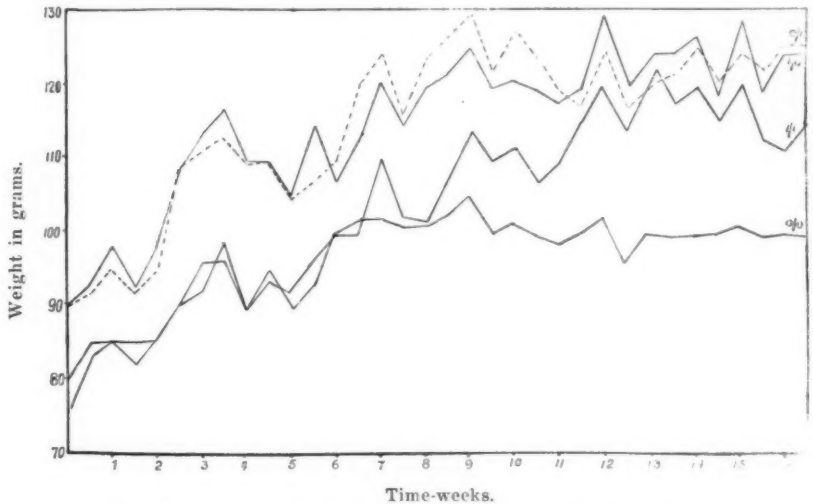


Growth-curves of rats from Series (2) on diet containing 2% tryptophane in the amino-acid fraction of the mixture. The shaded area represents the period during which the tryptophane was increased in amount to 3%, and it was maintained at that figure for Rat 1/0 until the point X.

It was found that within four weeks the synthetic diet was being almost entirely rejected, and that voluntary starvation, in whole or part, was playing a greater part than any deficiency in diet could do. The starch was reduced to 30% and the sugar raised to 27% to make a more palatable diet, and there

followed a temporary revival of appetite, which, however, soon failed again. Only by altering the manner in which the food was presented, e.g., in gelatin jelly variously flavoured, etc., could a full intake of food be assured.

The other experimental sets receiving added tryptophane, although evincing some distaste for the food, never completely rejected it, and careful preparation readily corrected any slackening in intake. This was attained by subjecting all food to the same manipulation as in the case of Experiment (4) in order to



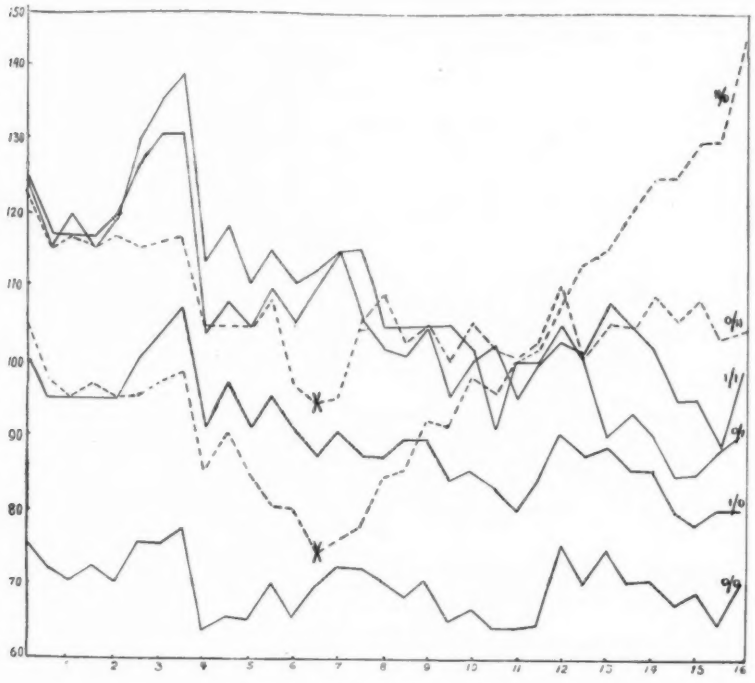
Growth-curves of animals from Series (3) on diet containing 0·5% tryptophane in the amino-acid fraction of the mixture.

maintain uniformity throughout. From the curves it will be noted that the growth failure is not so striking as was anticipated, and this may be due to the great care taken to ensure a nearly normal food intake, cf. Hopkins (2).

Two of the most debilitated animals were taken from the series and resuscitated by diet (3), great pains being taken to ensure their recovery by making the food as palatable as possible; unless this had been done these animals would certainly have succumbed. The fur was "glairy," the muscles limp, and the feet blue and numbed-looking. There was some incontinence, and the urine stained the fur an orange-yellow colour. The rectal temperature disclosed no deviation from normal, which, in the rat, is subject to a rather wide range of fluctuation.

At no time, however, was the tendency to spontaneous exercise absent, even though the tendency to huddle together, as though cold, was noted. The recovery

was so complete and surprising as to leave no doubt as to the absence of tryptophane being the cause of their previous failure in growth and maintenance. It was found that the series receiving 0·5% tryptophane in the amino-acid moiety of the diet did much better than those on 2%, their appearance more than their growth-curves illustrating this fact. When the amount of tryptophane was raised to 3% of the amino-acid fraction, these animals became markedly worse,

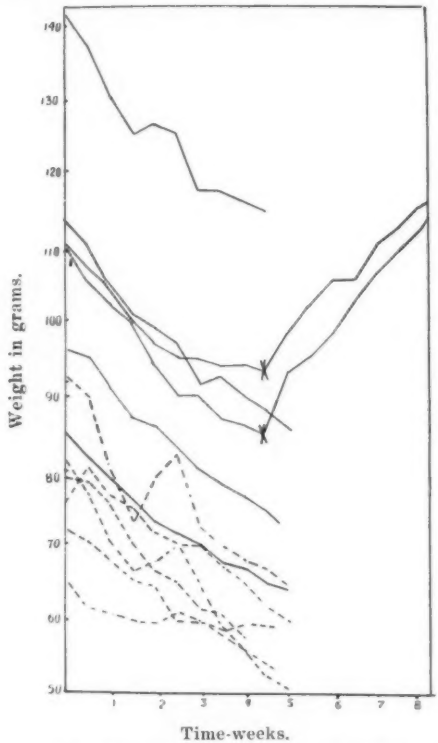


Growth-curves for animals from Series (4) on diet free from tryptophane. The dotted curves represent the growth of two animals to whose diet tryptophane was restored at X.

and resumed growth only on resumption of a 0·5% tryptophane diet. These animals looked quite like those in the early stages of feeding on a tryptophane-free diet. At post-mortem the animals on tryptophane-free diet showed complete absence of subcutaneous fat, although abdominal fat appeared normal in amount. The skin was parchment-like, and covered with sparse, matted hair, the rats resuscitated by restoration of tryptophane to the diet showing normal subcutaneous fat and a pliable skin covered with thick fur.

The histological changes observed in the thyroid glands will be discussed in a later paper.

Heat production was studied after the night's fast by the method of Prince (4), the animals being given a small dose of luminal sodium prior to the test (5).

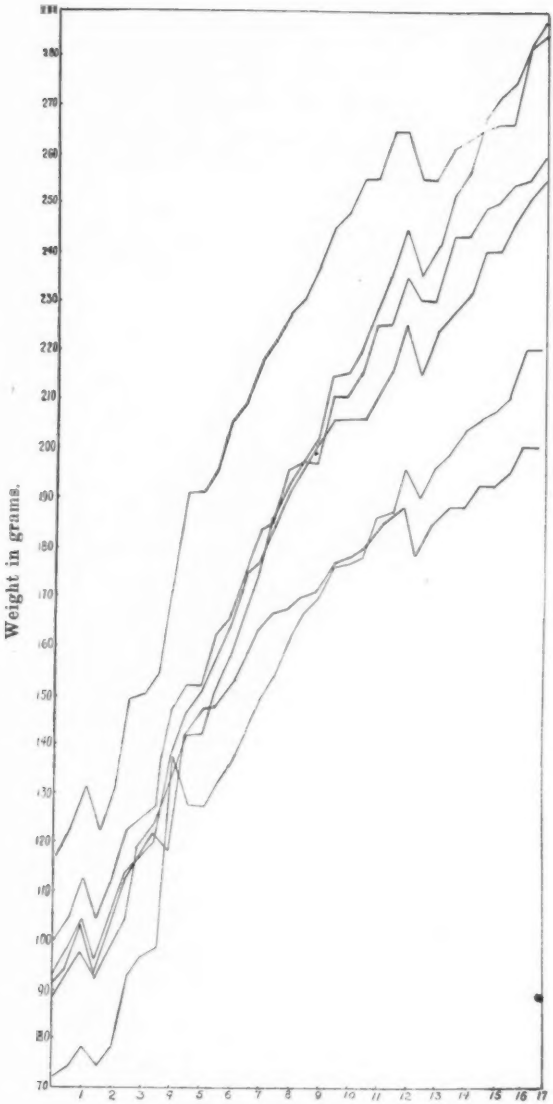


Growth curves from series of rats on Zein diet. Tryptophane was restored in two cases at X. Dotted curves are for females. All animals were moribund by the end of the fifth week.

The latter was made up in sterile saline, 0·08 grm. per cc. Dose, ·1 cc. per 200 grm. rat. This was enough to induce sleep if the animal was kept in the dark and undisturbed for some 15 to 20 minutes.[2] Results are given in terms of calories per unit surface area, calculated by the method of Krogh (5):

$$\text{Calories per kilogram} \times \sqrt[3]{\text{Weight in kilos.}}$$

[2]It is to be especially noted that the animals used were handled daily. They were exceedingly tame, and submitted to experimentation with no difficulty whatever.



Curves of growth of control Series (1). Diet of corn, bread and milk, and fresh carrots. All growth-curves are drawn to same scale, and show the great difference between normal and synthetic food "controls" over same period.

In the case of the second series of respiratory exchange determinations on rats of a Zein diet, the greatest care was taken to ensure standard conditions, owing to the results of the first series showing so small a deviation from normal. In order to illustrate the experiments, full numerical details are quoted for analysis. The unsatisfactory nature of the hydrolyzed casein diet led to a repetition of the work, using zein as protein supply, the diet being constituted as follows (6):

Zein 16·92%	
·54%	Lysine hydrochloride
·27%	Cystine
·26%	Na_2CO_3
26·34%	Potato starch
5·00%	Salt mixture
23·13%	Sucrose
15·00%	Butter
12·00%	Lard
½ cc.	Marmite extract

The growth-curves show a marked difference from the previous set. The failure to maintain weight is more marked, and despite the fact that no difficulty was experienced in ensuring that the diet was eaten, the duration of the experiment was reduced to one-third of that of the previous set. Using Tigerstedt's (7) figure of 227 calories per kilo of rat per 24 hours as a basal heat output, Krogh calculates the heat per unit of surface as 111. It will be seen that the basal heat output falls below normal in the case of the animals on tryptophane-free diet, but those receiving tryptophane are only relatively better in this respect, the figures giving no real indication of any specific effect. This could be due to the tissues supplying the needed amino-acid at their own expense, until death terminates the experiment, but it might be expected that some indication would be obtained, especially in the later stages. It might be pointed out that the rate of growth of the animals to whom tryptophane was restored is greater than those on full tryptophane diet throughout. The respiratory quotient and heat output follow the growth in this respect. The respiratory quotient of the animals on tryptophane-free diet points to metabolism of fat, or possibly, in the case of the lower figures, formation of carbohydrate from protein (8).

SUMMARY OF CONCLUSIONS.

1. Absence of tryptophane from a diet causes a falling off in heat production commensurate only with starvation wasting. There is no evidence from measurement of respiratory exchange that it is required for the elaboration of the thyroid hormone.

2. There is no fall in body temperature, even in the extreme stages of tryptophane-deficient feeding.

3. There is evidence that added tryptophane in a synthetic diet differs in its action from that combined in the protein molecule. It may cause toxic symptoms when increased beyond 2% of the protein fraction. The minimum amount of tryptophane necessary as such in a synthetic diet appears to be in the vicinity of 0·5% of the protein fraction. The optimal amount would appear to lie near the same figure.

4. Zein provides a better basis for such experimentation, the results of which, unless closely watched, are vitiated by voluntary starvation, particularly when hydrolyzed casein forms the basis of the diet.

From the results of the above long and carefully controlled experiments the latter would appear to be an unsatisfactory source of amino-acid, a fact not unconnected with the destructive action of the treatment upon the diketo-piperazine orientations pre-existing in the protein molecule.

Thanks are due to Professor Sir F. G. Hopkins for his advice throughout this work, which was carried out for the Medical Research Council during the tenure of a Beit Fellowship for Medical Research.

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TABLE 1.

Animals on Hydrolyzed Casein Synthetic Diet with Added Tryptophane.

Series (2).

Cage 4.

Date	Marks	Rectal Temp.	R.Q.	Weight Grms.	C x $\sqrt[4]{W}$ Per 24 hrs. *C = Calories per Kilo per 24 hrs. W = Weight in Kilos.*
9.11.25	0/1	—	0·78	95	84·0
9.11.25	11/0	—	0·68	152	79·2
11.11.25	11/0	—	0·76	148	76·8

Date	Marks	Rectal Temp.	R.Q.	Weight Grms.	C x $\sqrt[4]{W}$ Per 24 hrs. *C = Calories per Kilo per 24 hrs. W=Weight in Kilos.*
13.11.25	0/0	—	0·74	92	72·0
13.11.25	1/0	—	0·72	97	84·0
29. 4.25	11/0	96·7	0·68	154	86·0
29. 4.25	1/1	96·4	0·69	135	86·4
30. 4.25	0/0	95·0	0·70	105	79·2
30. 4.25	1/0	95·5	0·71	112	74·4
30. 4.25	0/1	94·8	0·73	90	76·8
30. 4.25	0/11	95·0	0·71	115	76·8

TABLE 2.

Animals on Zein Diet free from Tryptophane.

Cage 3.

Date	Marks	Rectal Temp.	Weight Grms.	R.Q.	C x $\sqrt[4]{W}$ Per 24 hrs. *C = Calories per Kilo per 24 hrs. W=Weight in Kilos.*
8.11.25	0/0	—	60	0·78	79·2
8.11.25	1/0	—	85	0·78	76·8
8.11.25	11/0	—	105	0·70	74·4
17.11.25	1/1	—	102	0·68	72·0
17.11.25	0/1	—	106	0·68	81·6
17.11.25	1/0	—	87	0·67	74·4
24.11.25	1/0	97·0	80	0·63	72·0
24.11.25	0/1	97·7	107	0·72	86·0
10. 3.25	0/1	95·6	100	0·68	74·4
10. 3.25	0/0	97·8	65	0·66	74·4
18. 4.25	1/1	97·4	95	0·69	76·8
18. 4.25	1/0	97·4	85	0·70	69·6
21. 4.25	1/0	97·4	78	0·68	62·4
21. 4.25	0/1	100·0	80	0·65	67·2

TABLE 3.

Experimental and Derived Data.

Tryptophane-free Zein Diet.

Time	Marks	Weight, Grams	Barometer	Total Volume Air Collected, cc.	Duration of Experiment	CO_2 %	O_2 %	CO_2 per Minute	O_2 per Minute	R.Q.	Air Temp., °C.	Calories per Kilo. per 24 hrs. = C.	C × $\sqrt[3]{W}$ (W = Kilos) (24 hrs.)	Date and Cage No.	
12.0	None ♂	94	757·3	6543	35	0·960	1·33	1·645	2·284	·72	22·9	164	74·4	27.11.25	K
11.15	0/11 ♂	100	777·5	6000	40	1·140	1·58	1·432	1·961	·73	22·2	132	61·3	5.12.25	K
12.15	0/1 ♂	118	775·5	6622	35	1·160	1·80	2·076	3·209	·65	21·9	165	81·0	5.12.25	K
4.0	1/1 ♂	95	760·5	6008	35	1·187	1·79	1·830	2·860	·63	20·2	202·8	92·5	7.12.25	K
4.0	0/0 ♂	86	752·6	6780	40	1·225	1·89	1·860	2·880	·647	20·85	175·2	77·3	8.12.25	K
12.30	1/1 ♂	101	755·5	7231	45	1·373	1·06	1·310	1·970	·77	21·8	131·5	61·2	9.12.25	K
3.30	11/0 ♂	80	755·3	5881	45	1·660	2·29	1·990	2·750	·72	21·6	185·0	86·0	10.12.25	K
12.0	11/0 ♀	68	758	5878	48	1·064	1·48	1·303	1·812	·718	23·0	179·6	73·3	11.12.25	L
11.30	1/0 ♀	68	772·2	6527	40	0·920	1·22	1·500	1·990	·750	23·6	197·3	80·5	16.12.25	L
11.30	0/1 ♀	90	771	6370	46	1·580	2·03	1·940	2·53	·76	23·7	189·5	84·9	17.12.25	L
11.30	0/11 ♀	58	765	5900	40	1·020	1·62	1·390	2·21	·63	23·6	255·0	98·9	18.12.25	L

Mean error for series C × $\sqrt[3]{W}$ = ± 10·7

CAN THE CATTLE TICK HAEMAPHYSALIS BISPINOSA ACT AS THE CARRIER OF PIROPLASMOSIS *(PIROPLASMA BIGEMINUM)*?

AN EXPERIMENTAL ENQUIRY

by

JOHN LEGG

(Government Veterinary Surgeon, Townsville, Queensland).

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The work, of which an account is given below, was undertaken at the request of the New Zealand Department of Agriculture, its object being to prove whether the cattle tick, *Haemaphysalis bispinosa*, which is widespread over part of the North Island of New Zealand, is capable of acting as the intermediary in the transmission of *Piroplasma bigeminum*, the causal organism of piroplasmosis of Queensland cattle.

So far as is known, *Haemaphysalis bispinosa* does not act as a vector in the spread of any known contagious disease of cattle, or for that matter of any other domesticated animal, on which it may be found in one or other of the stages of its life history. In this respect is differs from many other members of the Ixodoidea, and its only effect, though serious enough, is that of an external parasite.

In Queensland, on the other hand, is found *Margaropus annulatus* var. *australis*, another species of cattle tick, which is not only in itself a serious external parasite of cattle, but one that acts as the intermediary for *Piroplasma bigeminum*.

In years gone by, in Queensland the tick *Margaropus australis* gradually spread from the northern towards the southern coastal areas of the State; serious outbreaks of piroplasmosis occurred, causing a mortality as high as 95 per cent. amongst the highly susceptible cattle, and the effects of the tick as an external parasite at that time were relatively negligible. At the present moment outbreaks of piroplasmosis which occur in Queensland are practically wholly confined to areas of country until recently clean, into which the tick has made an incursion, or where cattle are removed without vaccination from clean into tick-infested areas.

The reason for the occurrence of so few serious outbreaks of piroplasmosis amongst cattle in the tick-infested areas of Queensland is the well-known one, viz., that calves soon after birth become infected with the piroplasm, and being highly resistant, develop early in life a strong immunity, or rather a tolerance to the disease, which toleration is being continually reinforced throughout the life of the animal through continual reinfection with ticks.

That the piroplasm exists in greater or lesser numbers in the blood of such animals is shown, firstly, by the fact that when the blood of such animals is inoculated, either subcutaneously or intravenously, into other susceptible animals, a reaction is frequently, though not always produced, with the appearance of piroplasms in the circulation of the latter. Secondly, we know that an animal which has recovered from piroplasmosis may suffer at some future date from another disease, and this latter may so lower the resistance of the animal that an increase of the piroplasms within the circulation follows, and piroplasmosis results. Such is a common experience with cattle reared in tick-infested areas in Queensland.

All Queensland cattle bred on tick-infested country, therefore, may be regarded as being tolerant to piroplasmosis, owing to infection when young, and the piroplasms may be regarded as being present in greater or lesser numbers in the circulation.

In New Zealand, on the other hand, the cattle tick, *Haemaphysalis bispinosa*, exists over a fairly wide area, but, as before stated, it does not act like *Margaropus annulatus* as the agent in the transmission of any known disease. This may be due, however, not to its inability to do so, but to the fact that the first of such ticks to enter New Zealand, and whose progeny is represented by the ticks at present there, may have been themselves "clean."

As New Zealand and Australia are countries not widely separated from each other, the question arises as to whether cattle which had spent some period of their lives in areas infected with *Margaropus annulatus*, and were thus infected with *Piroplasma bigeminum*, could with safety be transported to New Zealand, and into the areas infected with *Haemaphysalis bispinosa*, without setting up an outbreak of piroplasmosis. In other words, could *Haemaphysalis bispinosa* convey piroplasmosis, or redwater, as the disease is commonly called, in the same manner as *Margaropus annulatus?*

Comparison of the Life History of Margaropus annulatus with that of Haemaphysalis bispinosa.

A comparison of the life histories of these two ticks shows that one, *Margaropus annulatus*, is a continuous, and the other, *Haemaphysalis bispinosa*, is an interrupted feeder; in other words, one is a single host tick, and the other a three host.

The life history of the *Piroplasma bigeminum* in its stages through the tick, *Margaropus annulatus*, has not been worked out, but we know that the larvae raised from adults which have engorged on an infected or recovered animal are themselves infective. Neither has the biology of the tick, *Margaropus annulatus*, been fuliy worked out in Queensland, but so far as is known, the young larval ticks first moult about seven days after attaching to their host, and the second moult, from the nymph to the adult, takes place about the sixteenth day. These periods will possibly be influenced somewhat by the seasonal conditions prevailing at the time. Thus it has been found in America that the tick, *Margaropus annulatus*, may remain on its host up to 58 days, although the usual period, at least during the warmer months of the year, is 21 to 28 days. When the female *Margaropus annulatus* drops off, she may be found ovipositing within 4 days, and these eggs may hatch within 21 days (minimum), so that this tick would take at least 46 days to complete its life history from larvae to larvae. Under natural conditions it would probably be much longer.

Haemaphysalis bispinosa, on the other hand, is an interrupted feeder. It has been found under Queensland conditions to take at least 4 days to engorge and drop off, in each of the various stages of its life history, i.e., larvae, nymph, and adult, and at least 10 days to complete each of the two moults, so that to complete the period from unengorged larvae to engorged adult is at least 32 days. No figures are available to show the preoviposition period or the period of incubation of the eggs under Queensland conditions, but they are certainly not less than those of *Margaropus annulatus*, viz., 4 days and 21 days respectively. Thus it will be seen that even under artificial conditions the life cycle of *Haemaphysalis bispinosa* takes somewhat longer to complete than that of *Margaropus annulatus*. Under natural conditions, where both the newly-hatched nymph and the adult have to await their chances on the grass of attaching themselves to a passing beast, a circumstance which does not apply to a continuous feeder like *Margaropus annulatus*, it will be easy to recognize that *Haemaphysalis bispinosa* takes much longer to complete its life history than *Margaropus annulatus*.

Turning now to the question of the possibility of *Haemaphysalis bispinosa* acting as a transmitter of *Piroplasma bigeminum*, it will be noted, that being an interrupted feeder, it will be necessary to test the capacity of this tick to transmit the disease in any of the three stages of its life history by "infecting" the tick in the immediately prior stage. Even then, if the result were negative, there would still remain the possibility that the tick could convey the disease in a further subsequent stage. These latter possibilities are not likely with *Piroplasma bigeminum*, although we know that heartwater, as shown by Lounsbury, can be conveyed by the interrupted feeder, *Amblyomma hebraeum*, in the adult stage when it has fed on an infected animal in the previous larval stage, and on a non-infected or non-susceptible animal in the nymphal stage.

Keeping these possibilities in mind, a series of experiments were therefore outlined, in which the tick, *Haemaphysalis bispinosa*, was "infected" in each of the three stages (larvae, nymph, and adult), and then tested on non-immune animals in each of the three subsequent stages of its life history.

The experiments might be set out as follows:

"A" Series, where the ticks were engorged on an immune or recovered animal in the larval stage, then after moulting fed as nymphs on a non-immune animal (Experiment 1).

Young adults raised from the engorged nymphs from the last experiment were then tested on a second non-immune animal (Experiment 2).

From the engorged adults obtained from Experiment 2, larvae were raised, and these were allowed to engorge on a third non-immune animal (Experiment 3).

"B" Series, where the ticks were engorged on an immune or recovered animal in the nymphal stage, and then after moulting, fed as adults (Experiment 4) on a non-immune animal. From the engorged adults obtained from this experiment larvae were raised, and these were engorged on a second non-immune animal (Experiment 5).

From these engorged larvae nymphs were raised, and these were tested on a third non-immune animal (Experiment 6).

"C" Series, where the ticks were engorged in the adult stage on an immune or recovered animal, then after ovipositing and producing larvae, these were fed on a non-immune animal (Experiment 7).

From the engorged larvae obtained in the last experiment nymphs were raised, and these were engorged on a second non-immune animal (Experiment 8).

From the engorged nymphs obtained in the last experiment adults were raised, and these were engorged on a third non-immune animal (Experiment 9).

Details of Experiments.

In carrying out these experiments both immune and non-immune cattle were required. The former are common enough in the Townsville district, and as the tick is found for a distance of 200 miles to the west, it was found necessary to obtain the latter outside this area for the purpose of experiment.

In regard to the use of an immune animal bred on country infested with *Margaropus annulatus*, and using this animal for the purpose of "infecting" the ticks, *Haemaphysalis bispinosa*, it has been found that the citrated blood of many such animals, taken indiscriminately, frequently fails to invoke a reaction, even when injected in large doses into cattle highly susceptible to redwater. The cause of this inability is really unknown, because such cattle infected, as they

are, practically from birth by the tick, *Margaropus annulatus*, are highly resistant to piroplasmosis, but it is in all probability due to the scarcity of the piroplasm in the blood of such cattle, in spite of continual tick infestation. We do know that the blood of an animal which has recently suffered an acute attack of redwater, whether artificially produced or naturally acquired, is nearly always capable for some time after recovery of invoking a reaction in 100% of susceptible cattle, even though such animal be kept free of ticks *(Margaropus annulatus)*. This is as we would expect, but with piroplasmosis, as it occurs in Queensland, this ability is frequently soon lost, though the period varies in individual cattle, and it is probably due to the disappearance to a large extent of the piroplasms from the blood, and with the disappearance of the piroplasms (as tested by inoculation of susceptible cattle) there is frequently a loss of immunity. Cattle which have suffered a natural attack of piroplasmosis, and then kept free of ticks *(Margaropus annulatus)* for as short a time as twelve months, and again reinfected, have been known to suffer a second natural attack of the same disease.

Under the circumstances, therefore, it was thought advisable to use as an immune animal for the engorgement of the ticks *(Haemaphysalis bispinosa)* one that had been recently immunized, and whilst engorging the ticks *(Haemaphysalis bispinosa)* on this animal, to test its blood during the same period as often as possible by inoculation of susceptible cattle. This was found to be a feasible proposition, because susceptible cattle are continually arriving from the southern States for inclusion in the herds of North Queensland, and these latter animals come to the experiment station for vaccination purposes.

Another matter that required consideration was the necessity of confining the ticks *(Haemaphysalis bispinosa)* somewhere on the body of the animals, and preventing their escape, or else placing the animals under such circumstances that, if the engorged ticks did fall to the ground, they would not be in a position to infect another suitable host. It was also necessary to collect several batches of engorged ticks, sometimes from the one animal after they had dropped off, so that they could be allowed to moult, or in the case of the adult, reproduce eggs and larvae, to be again used for a further infestation.

This method of collecting the ticks in batches, and allowing them to moult under artificial conditions, was considered as likely to be conducive of more satisfactory results than allowing the ticks to fall off naturally to the ground, moult there, and then bring about natural reinfestation of non-immune cattle which had access to the area. This last method would also depend on the possibility of the tick adapting itself at once to Queensland conditions, and would be on the whole an entirely unsatisfactory and haphazard method; and so, acting on the suggestion of the Director-General of the New Zealand Department of Agriculture, it was decided to use bulls in all the experiments, and to confine the ticks as far as possible to the skin over the testicles, which were covered with

calico bags, so that engorged ticks on falling off would be caught and removed at leisure. This method proved to be entirely satisfactory, and the whole of the experiments have been conducted in this manner.

As the experiments were being conducted at Townsville, in the middle of country infested with *Margaropus annulatus*, it was essential that the non-immune cattle used should be placed under circumstances where infestation with this tick was impossible, and this was done by bringing the cattle by rail from non-infested country, and trucking them right into a paddock which had been cleansed of ticks *(Margaropus annulatus)* by systematic and continual dipping in the past. The bulls used in the various experiments were obtained on Afton Downs, country where the Queensland tick has never been known, and consequently cattle bred there are found to be highly susceptible to Redwater. These bulls have been kept for some months running in paddocks at the experiment station, and have never become infested with the Queensland cattle tick, and have remained in a condition of susceptibility to piroplasmosis.

Bull No. 1. This bull was selected for immunization, and passed through a typical redwater reaction in May, 1925. At first inoculation was carried out with blood from a locally bred animal, which had probably been infested with ticks all its life, and was certainly heavily infested at the time the blood was drawn, and on another occasion with blood from a second animal that had been kept free of ticks for some time, although originally infested. In neither case was a reaction produced, but the bull subsequently reacted to inoculation with blood from a third animal recently heavily tick-infested. Piroplasms appeared in the blood on the eighth, ninth, tenth, eleventh, and twelfth days after inoculation, and the ordinary clinical symptoms of piroplasmosis appeared, followed by recovery. This animal was then used during the subsequent six months for the engorgement of batches of *Haemaphysalis bispinosa*. During this time he was kept entirely free of *Margaropus annulatus*, but his blood was tested on the following dates in doses of 5 cc. in citrate solution.

Date.	Susceptible Animals Inoculated.	
15.7.25.	Cows 813, 814, 815, 815, 817	Typical reactions in all cases.
25.8.25.	Bulls 824, 825, 826	
7.10.25.	Bulls 838, 837, 839, 840, 841	

These were the only opportunities that occurred to test the blood of this bull on susceptible cattle during the period he was being used for the engorgement of batches of ticks *(Haemaphysalis bispinosa)*, but they are sufficient to indicate that his blood still contained the organism in sufficient numbers to invoke a reaction when inoculated subcutaneously.

Batches of larvae, nymphs, and adults *(Haemaphysalis bispinosa)*, all in the unengorged state, were required for engorgement on this animal. Some of these were obtained direct from New Zealand, but others had to be raised. To

raise these "non-infected" batches of ticks *(Haemaphysalis bispinosa)* the non-immune cattle were used.

Experiment No. 1. To test the capacity of nymphs, engorged as larvae on an immune animal (Bull No. 1), to convey piroplasmosis when fed on a non-immune animal.

4.9.25. Five nymphs from larvae which had engorged on Bull No. 1 were placed on Bull No. 3. These nymphs commenced engorging, but were all dead by 9.9.25. This experiment was then discontinued, as no more nymphs were available at the time. Bull No. 3 was under observation for the subsequent three weeks, but showed no signs of illness.

16.11.25. Infected Bull No. 6 with 70 nymphs. From this experiment over 50 engorged nymphs were obtained, the last being removed on 23.11.25. Temperatures and blood examinations were commenced on 23.11.25, and continued until 11.12.25. A perusal of the temperature chart of this animal shows that a fairly high temperature was maintained, but it must be remembered that the season of the year was early summer, and the bull was driven in each morning to have the temperature taken. In semi-tropical countries like North Queensland, high temperatures such as those shown on the chart of this bull are not unusual. The blood examinations were entirely negative. The animal was kept under observation a week after cessation of the taking of the temperatures and blood smears, and showed no clinical signs of redwater.

Experiment No. 2. To test the capacity of adults to convey the disease, having engorged on a recovered animal in the previous larval stage, then on a non-immune animal in the subsequent nymphal stage.

Adults raised from the engorged nymphs obtained in Experiment No. 1 were used in Experiment No. 2.

8.12.25. Fed 50 adults on Bull No. 4.

14.12.25. Some of the ticks have apparently escaped from the bag, owing to its being torn the previous night, when many were almost replete and ready to drop off.

Only 7 fully engorged and 5 partly engorged were removed on 15.12.25.

Temperatures and blood examinations were commenced on 15.12.25, and continued until 3.1.26. The result was entirely negative. The animal was kept under observation for a week following, and was found to show no signs of clinical redwater.

Experiment No. 3. To test the capacity of larvae to convey the disease, after having fed on an immune animal in the previous larval stage, then fed as nymphs on a non-immune animal, and secondly as adults on a second non-immune animal.

Larvae raised from engorged adults obtained from Experiment No. 2 were used for this experiment.

16.1.26. Infected Bull No. 4 with several hundred larval ticks raised from adults obtained from Experiment No. 2. The bull was reinfected with more

larvae on 21.1.26. Between 500 and 600 engorged larvae were obtained from this experiment, the last being removed on 25.1.26.

Temperatures and blood smears were obtained from this animal between 23.1.26 and 13.2.26, and showed no signs of a reaction.

"B" Series.

Experiment No. 4. To test the capacity of adults to convey piroplasmosis having fed as nymphs on an immune animal (Bull No. 1).

26.8.25. Fed 24 young adults on Bull No. 5.

1.9.25. Fed 15 young adults on Bull No. 5.

26.9.25. Fed 13 young adults on Bull No. 5.

From the above, 38 fully engorged adults were obtained, several others having been squashed by the animal lying down when the ticks were only partly engorged.

Temperatures and blood examinations were commenced on 2.9.25, and continued until 23.10.25. The result was entirely negative. Observation showed no clinical signs of redwater.

Experiment No. 5. To test the capacity of larvae to convey the disease, the parents having fed on a recovered animal in the previous nymphal stage, and then as adults on a non-immune animal.

Larvae raised from engorged adults obtained from the last experiment (No. 4) were used in this experiment.

13.10.25. Fed several hundred larval ticks on Bull No. 6.

15.10.25. All above larvae apparently dead by this date.

22.10.25. Fed several hundred larvae. Bull up to this date showed no signs of illness.

28.10.25. Fed a few hundred more fresh larvae.

6.11.25. From the above experiment about 900 engorged larvae were obtained altogether, and the attachment and engorgement of these ticks must be considered quite satisfactory. Last ticks were removed on 4.11.25.

Temperatures and blood smears commenced on 20.10.25, and continued until 19.11.25. The results were negative. The animal was kept under observation for a week following the cessation of temperatures and blood examinations, but no signs of redwater were evinced.

Experiment No. 6. To test the capacity of nymphs which had fed as larvae on a non-immune animal to convey the disease, the female parents having fed in their nymphal stage on a recovered animal, and then as adults on a non-immune animal.

Nymphs raised from engorged larvae from Experiment No. 5 were used in this experiment.

16.11.25. Infected Bull No. 5 with 100 nymphs.

From this infestation there were obtained 80 engorged nymphs, the last being removed on 23.11.25.

Temperatures and blood smears commenced on 23.11.25, and continued until 7.12.25. The result was negative. The animal was kept under observation for a further week. There was no sign of a reaction at any time.

"C" Series.

Experiment No. 7. To test the capacity of larvae to convey the disease, the parents having engorged in the adult stage on an immune animal.

26.9.25. Fed several hundred larval ticks on Bull No. 4.

30.9.25. All the above ticks apparently dead. None had engorged. Probably due to the hot weather.

1.10.25. Several hundred larval ticks placed on testicles.

5.10.25. Temperatures and blood examinations commenced. About 200 engorged larvae removed from bottom of bag.

13.10.25. Placed several hundred larvae on testicles.

15.10.25. Ticks placed on bull on 13th apparently dead. Infected with more larval ticks.

22.10.25. Many engorged ticks dead in bag. About 50 live engorged.

6.11.25. Again infected several hundred fresh larvae. From this last infestation about 400 engorged larvae were obtained, the last being removed on 11.11.25.

From this experiment about 850 engorged larvae were obtained.

Temperatures and blood examinations continued until 28.11.25. These were entirely negative, and further observation of this bull indicated no sign of a reaction.

Experiment No. 8. To test the capacity of nymphs to convey the disease, the parents having fed in the adult stage on a recovered animal, and the larvae on a non-immune animal.

Nymphs raised from the engorged larvae obtained from the last experiment (No. 7) were used in this experiment.

27.10.25. Fed 31 nymphs on Bull No. 3.

From the above experiment 23 engorged nymphs were obtained. Some nymphs had escaped from the bag. Last ticks were removed on 2.11.25.

Temperatures and blood smears taken from 2.11.25 to 22.11.25 were entirely negative.

The above experiment was then repeated as follows:

23.11.25. Fed 250 nymphs on Bull No. 3.

From the above 86 engorged nymphs were obtained, the last being removed on 28.11.25.

Temperatures and smears taken from 30.11.25 to 14.12.25 were negative. The bull was kept under observation for another week, but evinced no signs of a reaction.

Experiment No. 9. To test the capacity of adults which, in the larval stage had engorged on a non-immune animal, and as nymphs on a second non-immune

animal, to convey the disease, the parents having engorged in the adult stage on an immune animal.

Adults raised from the engorged nymphs obtained from the last experiment (No. 9) were used in this experiment.

8.12.25. Fed 80 adults on Bull No. 5.

From this experiment 53 engorged adults were obtained, the last being removed on 17.12.25.

Temperatures and blood smears were commenced on 15.12.25, and continued until 3.1.26.

There was no sign of a reaction at any time. The animal was kept under observation for a further week.

DISCUSSION.

A perusal of these experiments, which were all negative, indicates at once that the most important are Nos. 1, 4, and 7, whilst possibly some importance might be ascribed to Nos. 2, 5, and 8, but it is scarcely likely that positive results could be expected from Nos. 3, 6, and 9.

It seemed, then, that all that was now required to be done was to test the susceptibility of the various non-immune bulls used for engorgement of the "infected" ticks.

However, this was not done at once, the reasons being as follows:

It has already been pointed out that the blood of immune Bull No. 1 was tested as often as possible for virulency during the six months over which it was used for "infection" of the ticks *(Haemaphysalis bispinosa)*, and was found on all occasions to invoke a reaction in susceptible cattle. These were the only cattle available at the time, and they were all bulls or cows imported for stud purposes from areas non-infested with *Margaropus annulatus*.

This seemed proof enough that the blood on which these ticks *(Haemaphysalis bispinosa)* engorged was infected with piroplasm, but it must be remembered that ticks only engorge a relatively small quantity of blood, especially larvae and nymphs, as compared with the amount of blood used for inoculation purposes (5 cc.). The possibility must therefore be borne in mind that a large number of ticks raised on this bull (No. 1) might not carry any, or enough, of the piroplasm to convey the disease, because, relatively speaking, we were only dealing with a small number of ticks in each experiment.

We do know, of course, that some tick-borne diseases can be conveyed experimentally by one tick, and theoretically this should be sufficient with piroplasmosis in Queensland. But with *Margaropus annulatus* in this State, experience suggests that a large number do not carry the piroplasm, because the tick has been known to appear in a previously non-infested area, and amongst

non-immune cattle, and yet piroplasmosis has not followed, at least, not immediately. It has been assumed that the appearance of such "clean" ticks in previously infested areas has been brought about through the agency of horses, because *Margaropus annulatus* can, and does occasionally, infest and reach maturity on both horses and sheep, and it is believed that the larval tick on biting the horse "cleanses" itself, and that its progeny are clean, i.e., free from piroplasms. It is probably quite correct that the larvae raised from adults engorged on horses do not produce piroplasmosis when placed on susceptible cattle, but there is another reason altogether by which we can probably account for the introduction of ticks into previously non-infested areas *without accompanying piroplasmosis*.

It has been stated earlier in this paper that there is evidence to show that the blood of Queensland cattle bred on tick-infested country, and more or less heavily infested with ticks all their lives, will frequently fail to invoke a reaction when inoculated into susceptible cattle, and the suggestion is put forward that this is due to the small number of organisms in their blood. In fact, the small number of experiments carried out by ourselves shows that the majority of such animals are quite useless for bleeding purposes, as their blood is quite avirulent, even in fairly large doses. When it is remembered that the tick engorges a small amount of blood compared with that used with a hypodermic syringe, it is quite easy to understand why so many of their progeny will be non-infective. This, of course, is assuming that the piroplasm does not multiply, or if so, at least not to any considerable extent, in the body of the tick. We have no direct evidence on this point, because we do not know the life history of the piroplasm, neither have any experiments been conducted to test the capacity of small numbers of the larvae, the progeny of adults raised on such animals, to convey the disease in Queensland, because susceptible cattle are not easily available, at least, where we are situated. But we have other evidence to support our view. We know that butchers' cattle, brought in from clean country to the tick-infested country on the coast, frequently do not show evidence of piroplasmosis for some weeks after being placed in paddocks on the coast, even though they become tick-infested within a day or so of their arrival. This idea is further borne out by many of the older inhabitants, who remember the first invasion of the ticks, and who state that the tick frequently appeared some time before the accompanying redwater.

In a report written by Dr. Sidney Hunt, Government Pathologist, to the Chief Inspector of Stock for Queensland, concerning experiments which he (Dr. Hunt) had carried out on redwater, reference is made to certain Boolburra cattle used in the experiments. The report says: "These cattle (Boolburras), though grossly infested for some time, had never had Texas fever amongst them, although they were themselves (as was afterwards shown) as susceptible to that disease as clean cattle."

It cannot be regarded as presumptious to assume that many of the ticks

which occur in the tick-infested areas of Queensland are not infected with *Piroplasma bigeminum*, simply because the organism is so rare in the blood of cattle immune to piroplasmosis, and it is such ticks, at first probably introduced in very small numbers, that are really responsible for the outbreaks of ticks, without accompanying piroplasmosis, in fresh areas. There is therefore no need to assume the spread of such ticks by means of horses. That cattle can easily wander across quarantine lines, which are not even properly fenced, or have no natural boundary, in some of the sparsely settled areas of Queensland, needs no further comment.

It might be possible, therefore, that although every precaution was taken to test the blood of the immune bull (No. 1), the reason for the failure of *Haemaphysalis bispinosa* to convey the disease was that the ticks were not picking up the infection, as it were, in sufficient quantities at least, to convey the disease to the non-immune animals. This possibility would not be so great with this particular animal (No. 1), because his blood was proved infective on all occasions used, whereas the blood of many animals bred and reared on tick-infested country in Queensland, and tick-infested all their lives, is frequently quite avirulent, even in large doses.

Still the amount of blood from Bull No. 1 used in each inoculation would be equal to that picked up by a fairly large number of ticks, and probably more than that picked up by the greatest number used in any experiment.

The best method of further testing the whole question was to repeat those experiments likely to prove positive, using immune animals, other than the particular one used in the experiments already concluded, for the engorgement of the ticks. A second bull (No. 2) was therefore immunized. It was inoculated with 10 cc. blood from Bull No. 837, and passed through a typical reaction. Piroplasms only appeared in the blood on one day, the twelfth, and could not be found at any other time, even after prolonged searching. On the fourteenth day, nymphs and adults were placed on the testicles of this bull, and with the engorged nymphs and adults obtained Experiments Nos. 4a and 7a were repeated. These were simply repetitions of Experiments Nos. 4 and 7.

Experiment No. 4a.

5.1.26. Fed 12 young adults on Bull No. 6.

9.1.26. Fed 20 young adults on Bull No. 6.

From the above experiment 11 fully engorged adults were removed, the others being partly engorged and squashed in the bag. Last ticks removed 16.1.26.

Temperatures and blood examinations between 12.1.26 and 30.1.26 showed no signs of a reaction.

Experiment No. 7a.

16.1.26. Infected Bull No. 5 with larval ticks.

Daily examinations showed that a number of ticks escaped from the bag at the neck. On 18.1.26 the calico bags were found off this bull. On 20.1.26, 23 engorged larvae were removed. On 21.1.26 this animal was reinfected with several hundred more larvae. On 25.1.26 50 more engorged larvae were removed. Although but few ticks were actually found in the bags, the engorgement of ticks on the animal was quite satisfactory. A rough estimate would be 500 to 600 engorged larvae. Many were removed in a partly engorged state, and many others must have fallen into the bedding.

Temperatures and blood examinations were taken between 23.1.26 and 15.2.26, and proved negative.

After these experiments had proved negative, an attempt was made to immunize a third bull (No. 3), but this animal died on the fourteenth day after inoculation from acute piroplasmosis, when about 20% of the red blood corpuscles were invaded with piroplasms, and before any ticks could be raised on this animal.

A further attempt was then made to immunize a fourth bull (No. 6). Piroplasms appeared in the blood on the ninth, tenth, eleventh, twelfth, and thirteenth days after inoculation, and on the twelfth day a batch of nymphs was placed on the testicles, and commenced to engorge, whilst piroplasms were still demonstrable in the blood by microscopic examination. These engorged nymphs, after moulting, were then used to repeat Experiment No. 4 (Experiment No. 4b).

Experiment No. 4b.

24.3.26. Infected Bull No. 5 with 12 young adults. Nine of these fully engorged, and three only partly engorged, the last being removed on 7.4.26.

Temperatures and blood smears were taken until 12.4.26, and the animal subsequently kept under observation. There was no sign of a reaction.

It was hoped to raise batches of larvae adults on this bull to repeat Experiments 1 and 7, but these all died before they reached maturity, although they commenced to engorge. The weather was exceedingly hot at the time, and the ticks seemed to object particularly to confinement within the bags during hot weather. Before other batches could be engorged this bull also died as the result of the inoculation. Incidentally, the reactions followed by death, which Bulls Nos. 3 and 6 gave to inoculations of blood from a recently recovered animal, showed their susceptibility to piroplasmosis at the time they were used for previous experiments.

Only two non-immune animals (Nos. 4 and 5) remained, and as there was little prospect of obtaining others, and as they had been used in some of the previous experiments as non-immune animals, it was necessary to test their susceptibility. Besides, the drought conditions prevailing at the time precluded

any possibility of obtaining further animals for experimentation. These two bulls were therefore each inoculated with 10 cc. blood from a recently recovered animal, and both passed through typical reactions.

CONCLUSIONS.

1. The engorgement of *Haemaphysalis bispinosa* in each of the different stages of its life history on an animal recently recovered from piroplasmosis, followed by engorgement on a different susceptible animal in each of the three subsequent stages of its life history, failed to convey the disease.

2. All the susceptible cattle subsequently reacted to inoculation with virulent blood.

3. The experiments point against the possibility of *Haemaphysalis bispinosa* acting as the intermediary in the spread of bovine piroplasmosis *(Piroplasma bigeminum)*.

THE MILK OF THE MONOTREME—*ECHIDNA ACULEATA MULTI-ACULEATA*

by

HEDLEY R. MARSTON

(From the Darling Laboratories of Physiology and Biochemistry, University of Adelaide).

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The most primitive milk-producing animals existing are the monotremes, the only two genera of which are represented by the Echidnidae (native porcupines or spiny ant-eaters) and the Ornithorynchidae (the duck-billed platypus). They possess the essential structural characteristics of the mammalia. Their skeleton is distinctly mammalian, though with certain reptilian reminiscences, especially in the limb girdles, and the reptilian condition is still preserved in the fact that the genito-urinary system and the rectum open into a common cloaca.

The monotremes retain the most striking traces of their proximate ancestry in their mode of reproduction, as they lay eggs, which they hatch out either in a nest or an incubatorium pouch, instead of nourishing the foetus in utero by means of a placenta.

The Echidna transfers the egg to its ventral pouch, which in the breeding season becomes a functional incubatorium, where it very soon hatches, and the embryo obtains its nourishment by nuzzling the milk secreted from the many openings of the mammary ducts, which are scattered over the surface of the breast in the region of the pouch (1). As there is no development of a definite teat in the monotremes, it is practically impossible to collect milk from the living animal. The specimen from which the milk was obtained came from Rocky River, Kangaroo Island, and was of the species *Echidna aculeata multi-aculeata* (Rothschild, 1905).

The young Echidna was removed from the pouch on October 1, and the animal killed by chloroform fourteen days later. One-half hour after death, on incising the gland on one side, a considerable quantity of milk exuded, whereupon Professor F. Wood Jones suggested that a sample might be collected from the other side. The skin was incised and lifted, exposing the mammary gland. The capsule was then incised, and the contents squeezed towards the opening, where the milk was collected by suction, with little, if any, contamination with blood; 8·5 cc. in all were collected.

Methods of Analysis.

The *specific gravity* was determined in a 5 gm. S.G. bottle, and was 1·023.

The *proteins* were separated, and analyzed as follows:

1. *Casein.* One cubic centimetre (by means of an Ostwald pipette) was diluted to 5 cc. with distilled water, and ·02N citric acid added drop by drop until the casein flocculated; 2 cc. of phosphate-citrate buffer at P_H 4·7 were then added, and the sample made up to 10 cc. It was then centrifuged at high speed for 30 minutes, the supernatant liquid separated, and the nitrogen content of 1 cc. estimated by the micro method of Folin. The precipitate was soluble in dilute alkali, and precipitated from solution at a hydrogen-ion concentration approximating $10^{-4\cdot7}$, i.e., about the iso-electric point of the casein of cow's milk. Owing to the smallness of the sample available, it was impossible to further identify the nature of this protein fraction, but it is a reasonable assumption to class it as casein.

2. *Albumin and Globulin Fraction.* Five cubic centimetres of the supernatant liquid from the casein estimation was sealed in a hard glass tube, and immersed in a boiling water bath for two minutes. The tube was quickly cooled, and the contents transferred to a stoppered centrifuge tube, and spun at high speed for half an hour. The nitrogen content of this supernatant liquid was determined. Total nitrogen of a 1·10 dilution of the whole milk was similarly estimated. The nitrogen content of the different fractions was calculated by differences:

	Nitrogen per 100 cc. of Undiluted Milk.
Total	2·12 grms.
Total—Casein	0·92 grms.
Total—Casein—Albumin, Globulin (that is, non-protein N.)	0·34 grms.

3. *Carbohydrate.* One cc. milk was diluted to 10 cc., 2 cc. of ⅔ normal H_2SO_4 was added, and 2 cc. of 10% sodium tungstate, and the whole made up to 20 cc., and filtered. The filtrate was treated in a similar manner to a blood filtrate in the new Folin method for estimation of blood sugar. It was, however, read in the colorimeter against the colour produced by the reducing action of a standard lactose solution prepared from purified and desiccated lactose. The content of lactose was 2·81%.

4. *Identification of Carbohydrate.* The protein-free filtrate was added to the supernatant liquids from the protein analysis, boiled, filtered, and concentrated to about 2 cc. on a water bath; 2 drops (⅕ cc.) of phenylhydrazine base were added, together with 2 drops of glacial acetic acid, and the mixture placed in a boiling water bath for 30 minutes. It was then allowed to cool. The osazone was separated by centrifuging and recrystallized from dilute alcohol. The microscopic appearance of the osazone was definitely that of lactosazone.

5. *Fat.* The fat was determined by extraction of 2·6336 grms. milk absorbed in a fat-free extraction thimble with ether in a Soxhlet apparatus. The dried fat was determined gravimetrically. It consisted of a colourless oil, and occurred to the extent of 19·62%.

6. The *Saponification Value* of the fat was determined by the addition of 20 cc. of standard ·2M alcoholic KOH directly to the fat (·8168 gms.) in the Soxhlet flask, and refluxing for 30 minutes, back titrating with ·05N sulphuric acid. The saponification value proved to be ·192, practically the theoretical value for pure triolein.

7. *Soluble Volatile Fatty Acid* was estimated by completely precipitating the fatty acid from the soap formed in the previous test with excess of ·05M H_2SO_4, 200 cc. of water was added, and the mixture distilled through an adequate trap and condenser until 110 cc. of distillate had been collected. This was filtered from the insoluble fatty acid, and 100 cc. of the filtrate titrated with ·05M KOH. The titration value was less than one drop of alkali of this strength, showing the absence of soluble volatile fatty acid.

The fat was almost certainly pure triolein. The fatty acid was liquid at the temperature of the laboratory (20°C.), and melted at approximately 15°C.

Total solids and ash were determined gravimetrically with the rest of the available sample by drying at 100°C. in a silica dish, and later ashing in a furnace. The total solids by estimation were 36·75%, and ash ·78%.

TABLE A.

	Specific Gravity.	Protein: Casein.	Protein: Albumin. Globulin.	Non-Protein Nitrogen.	Lactose.	Fat	Sapon. Value of Fat.	Sol. Volatile Fatty Acid of Fat.	Ash.	Total Solids	Total Solids. accounted for.
Echidna	1023	8·4	2·9	0·34	2·81	19·62	192	Nil	0·78	36·75	34·85
		Total Protein.									
Rabbit (3) ..	1047	12·0		—	2·00	13·5	—	—	2·50	30·5	—
Man (3)	1029	0·80	1·21	—	6·37	3·74	—	—	0·30	12·48	—
Cow (3)	1031	2·88	0·51	—	4·94	3·68	220–230	25–30	0·72	12·73	—

DISCUSSION.

It will be noticed, on referring to Table A, that the milk contains essentially the same constituents as that of the higher mammals. The main protein is undoubtedly a casein. The carbohydrate is the disaccharide lactose. It is together with the differentiation of the mammary gland tissue that lactose makes its appearance in nature. Galactose, one of its constituent hexoses, occurs in combination in the brain of the higher vetebrates, and almost certainly also occurs in similar nerve tissues of the Reptilia. The fact that lactose is a non-threshhold substance, and is passed out without reabsorption in the kidney,

whereas glucose (the normal sugar of the blood) is reabsorbed, makes it possible that lactose is the only sugar available to the organism which could be passed in concentrated form through the cells of the secreting acini.

The fat is peculiar, differing from that of the cow (the milk fat which has been most studied) in that it evidently consists of pure triolein, and contains no triglycerides of the lower fatty acids (tributyrin, etc.). The high content of fat is extraordinary when compared with that of the milk of man and of the cow, and it becomes evident from the ratio of carbohydrate to fat in the milk that the young Echidna must either store fat during the whole of the lactating period, or be endowed with the extraordinary power of α-oxidation of fatty acids. Otherwise extreme acidosis would follow from the accumulation of β-hydroxy butyric acid and other acetone bodies.

The baby rabbit is faced with the same predicament.

Assuming after Shaffer (2) that protein produces 20 millimols of anti-ketogenic substance and 10 millimols of diacetic acid per grm. of nitrogen, that fat produces $\frac{1}{2}$ mol of glycerol and 3 mols of diacetic acid per molecule of fat (the actual amount per grm. may be obtained from the saponification value of the fat in question), and that carbohydrate gives 5·56 millimols of antiketogenic substance per grm. of glucose, the approximate ratios of the milks shown in Table A are as follows:

Echidna	..	..	Anti : Keto :: 1 : 2·05
Rabbit	..	..	Anti : Keto :: 1 : 2·2
Man ..	..	..	Anti : Keto :: 2·1 : 1
Cow ..	..	..	Anti : Keto :: 2·5 : 1

In both the Echidna and the rabbit the complete oxidation of the milk-fat is impossible without the production of aceto-acetic acid in excess in the organism, and consequent acidosis.

SUMMARY.

The milk of Echidna does not differ in any extraordinary way from that of the higher mammalia; its constituents are the same, and their ratios fall within the limits of those found in the milks of other mammals. The absence of glycerides of soluble volatile fatty acids from the milk-fat is the only peculiarity which does not appear to have been noted in other milks.

I desire to thank Professor F. Wood Jones for his courtesy in collecting the milk and presenting the sample to the Department of Biochemistry.

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THE EFFECT OF A CONTINUED POLYURIA UPON THE KIDNEY OF THE RAT

by

C. S. HICKS AND M. L. MITCHELL

(From the Biochemical Laboratories, University of Cambridge).

(Submitted for publication 13th November, 1926.)

In the course of a study of inositol excretion, Needham (1) produced extensive polyurias in rats, amounting at a maximum to 140 cc. of urine per rate per 24 hours. His animals survived 14 weeks, and at autopsy the kidneys were found to be completely disorganized. It was felt that the evidence for the latter contention was insufficient, and the symptomatology appeared to be unaccounted for by the findings, and in order to study the phenomenon, as well as to investigate the kidney function more closely, experiments were carried out as follows: Twelve adult rats were sustained on a normal balanced dietary of casein, potato, starch, butter, sucrose, and salt mixture, plus marmite, and to the mixture was added an excess of common salt, which was slowly increased until 15% was being ingested.

Hopkins' metabolism cages, holding six rats comfortably, were provided with a continuous water supply at two of the four chambers, and in the remaining two, packed tightly, and slightly moistened to assist binding, was placed the food. In this manner the animals could feed but not scrape the powdered food mixture into the cage, and so contaminate the urine. This apparently unpalatable diet was eaten with avidity, and only when the added salt reached 15% did one or two of the rats go off their food temporarily. Water was consumed continually, and a marked polyuria resulted. The faeces were separated from the urine by the usual glass bulb separator, and only in the extreme degrees of polyuria did they become soft and adhere to the collecting funnel or separator in any degree.

The appended chart shows the extent of the polyuria attained, and the point to be noted is the rapidity of readjustment following on resumption of a diet containing no excess salt, but identical in all other respects with the diet that preceded the change. There is some evidence that in the later stages the adjustment is not so rapid when the chemical constituents of the urine are examined, but the volume adjustment appears to be unimpaired so far as these experiments

have gone. A long and detailed study of the chemical constituents and nitrogen partition has been made by one of us (M.L.M.), and will be reported later.

The animals not only maintained weight and condition, but also grew. There was no sign of oedema, either macro- or microscopically, nor by a study of weights after ceasing the salt diet, in fact, the rapid urinary adjustment corroborates this. The ratio of nitrogen/chlorine per cc. remained constant within the limits of error, indicating that fluctuations in dilution followed merely on fluctuations in food intake. There was no albumin in the urine at any stage. In order to prepare organs representing the condition of affairs at any stage in the experimental period, animals were killed and the tissues fixed in the following solution (2):

Potassium Bichromate, 40 grms.
Chromium Fluoride, 40 grms.
Water (distilled), 2,000 cc.

Sections were cut and stained by means of Mallory's phosphotungstic acid haematoxylin method, which has the advantage of showing up in a very marked degree certain granular appearances in the cytoplasm of the cells of the convoluted tubules (3). Others were stained by Scott's method (4), and do not show this feature, but show the absence of any interstitial change which might be expected after so long a period of strain. As may be seen from a study of the micro-photographs, there is no evidence that "the whole picture is one of enormous nuclear degeneration, together with general breakdown of cells," and all that one can say is that the cells are somewhat swollen and in a condition of great activity. The granules stained by the Mallory method show an interesting distribution almost entirely in the cells of the convoluted tubules, and, moreover, in these are packed densely towards the basement membrane of the cell. In the section from a kidney taken after the urine had returned to normal, these granules are seen to be distributed throughout the cytoplasm, and in most cases an arrangement of the granules in rows perpendicular to the basement membrane can be made out. These are undoubtedly the rows of fine granules forming the striations noted by Suzuki (5). According to Aschoff (6) the carmine injected in Suzuki's experiments did not stain these granules, but lay between the rows of granules prior to the final regression phenomenon of vacuole formation. These phenomena were interpreted as evidence against the reabsorptive activity of the cells, but recently Aschoff leans towards Mitamura's interpretation, the latter having shown that if in the rabbit a polyuria is induced in which secretion is increased 10 times, there is no deposit of carmine in the tubule cells, owing to the rapid passage of the urine down the tubules, the dye being present, however, by filtration through the glomerular capsule (7). In the present experiments, the mere concentration of the granules under the stress of the intense polyuria, in which secretion is increased 25 times, points to the passage of fluid towards the basement membrane. It will also be noted

that the tubes of Henle's loop are quite abnormally dilated, and this the more so by comparison with their fellow convoluted tubules. This may have a bearing on the possible pressure regulating mechanism suggested by various American and German authors (8), for it would appear that there is a collection of fluid in this situation between the primary and secondary convoluted tubules.

It is also evident that the glomeruli in extreme instances show evidence of some compression, and the interstitial spaces are wider than normal, the whole organ being very vascular. The liver shows evidence of this increased vascularity, as might be anticipated. It was friable, and crumbled when handled by the fingers in some cases, and in all cases weighed twice the normal weight. That this enormously increased flow through the portal area might have effects on liver function is to be expected, and there is evidence in favour of an increase in blood ammonia, which, owing to variations which seem to be related to the extent of hypertrophy of the liver, is at present under further investigation. The rest of the portal absorption area, apart from vascularity changes, shows little microscopic change, except the liver, where some increase of portal supporting tissue is evident, the epithelium and sub-epithelial tissue of the gut varying little, if any, from normal. When the rate of absorption and final excretion is considered in terms of the diameter of the tubes through which the fluid passes, the compensatory effort is the more to be appreciated. An interesting reflex of this is seen in the definite hypertrophy of the left heart.

SUMMARY AND CONCLUSIONS.

1. An experimental sustained polyuria of an extensive degree has been studied.

2. Over an experimental period of four months no evidence of impaired kidney function has been obtained.

3. Histological examination of the kidney shows no pathological change, all alteration being within the limits of normal.

4. The arrangement of cytoplasmic granules, staining by phosphotungstic acid haematoxylin, suggests active resorption from the lumen of the convoluted tubules towards the base of the living cells.

5. The livers of the experimental animals increased to double the normal size and weight, due to increased vascularity and possibly cell hypertrophy.

6. There is evidence of disturbance in urea formation, the blood ammonia being increased in the four animals so tested to six times the normal figure.

Acknowledgment is due to Dr. G. Scott Williamson, of the Dunn Laboratories, St. Bartholomew's Hospital, for assistance in preparing some of the sections; and to Dr. T. N. Seth, of Cambridge, for the analyses of blood for ammonia content.

This work was carried out during the tenure of one of us (C.S.H.) of a Beit Memorial Fellowship for Medical Research.

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Plate 1 shows section of cortex from rat passing 120 cc. urine per 24 hours after three months' poluria. Note the linear arrangement of the granules of the cytoplasm in the cells of the convoluted tubules and their deeper concentration towards the basement membrane. Compare the distension of the descending loop of Henle at X with that of the active associated convoluted tubules, where the lumen is more apparent than real, owing to the transparent nature of the cytoplasm as stained in this manner. The cell borders in the case of the "loop" epithelium are well defined, however, thus indicating less (if any) effect upon the latter than the former. Stained by phosphotungstic acid haematoxylin. Magnified ca. 300 diameters.

Plate 2. High-power view of section shown at Plate 1, giving in greater detail the indistinct nature of the cell border and its subjacent cytoplasm as stained by this method. The photograph is to be compared with Plate 8, in which there is some recovery from the effects of the polyuria. Stained by phosphotungstic acid haematoxylin. Magnified ca. 600 diameters.

Plate 3, showing cortex from polyuric rat during polyuria after four months' experiment. The method of staining shows the granules seen in the Mallory stained sections as white spaces. The cytoplasm generally takes up the stain, and the "brush" border cannot be distinguished as such. The distension of the descending limbs of Henle's loop can be compared with that of the convoluted tubules. The increased vascularity may be noted, as in the glomerular section. There is no increase of interstitial connective tissue. Stained by Scott's standard haematoxylin. Magnified ca. 600 diameters.

Plates 4 and **5.** High-power views from cortex of kidney from normal and polyuric rats respectively. Compare the distribution of granules in the cytoplasm of the convoluted tubules. Note that though the density of the photographs differs, the concentration of granules is greater towards the periphery of the polyuric tubular cells. Phosphotungstic acid haematoxylin. Magnified 400 diameters.

Plate 6. Section to show blood-vessel and interstitia. A glomerulus appears at X. The descending Henle loops are well seen to be dilated. There is no increase of interstitial tissue. Stained by Scott's standard haematoxylin. Magnified ca. 600 diameters.

Plate 7. Portal area from liver of rat after four months' polyuria, during active polyuria. Showing vascularity and some increase in portal interstitial tissue. The portal vein is dilated and engorged. The liver cells show no alteration either in cytoplasm or nuclei. Stained by phosphotungstic acid haematoxylin. Magnified ca. 150 diameters.

Plate 8. Section of cortex of kidney from experimental polyuric rat seven days after return to normal secretion after two months' experimentation. The dark staining granules are more widely distributed through the cytoplasm, and are not so concentrated towards the basement membrane. Scott's standard haematoxylin. Magnified ca. 600 diameters.

Plate 9. General view of normal rat kidney, showing selective action of stain upon the convoluted tubules. Compare with Plate 10 from polyuric rat kidney in fifth month of activity. Phosphotungstic acid haematoxylin. Magnified ca. 100 diameters.

Plate 10. General view of polyuric rat kidney in fifth month of activity. Note increased interstitial spaces and glomerular capsule space; other spaces not more dilated than normal. Staining and magnification as in Plate 9.

Plate 11. Showing a glomerulus and capsule from a rat at the height of polyuria after four months' experiment. The glomerular tuft will be noted to be very vascular, the half-tone discrete bodies being corpuscles. The intra-capsular space is not much greater than normal, and the capsule is not thickened. Scott's standard haematoxylin. Magnified 600 diameters.

Plate 1.

Plate 2.

Plate 3.

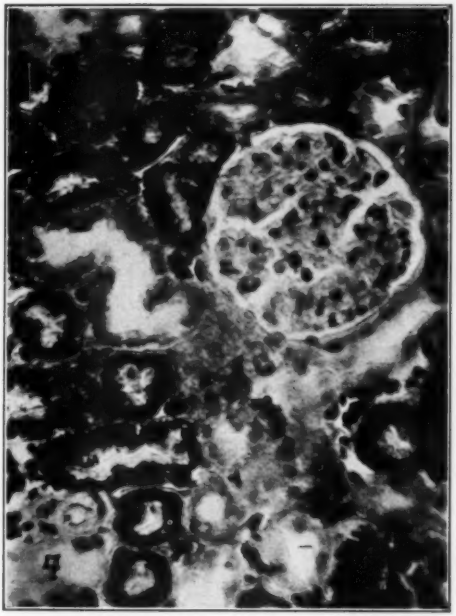

Plate 4.

Plate 5.

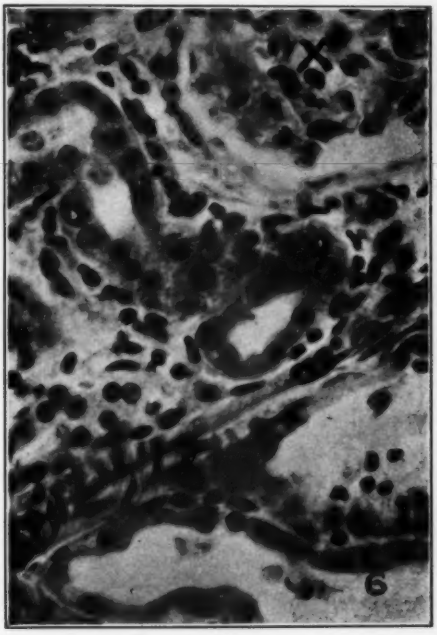

Plate 6.

Plate 7.

Plate 8.

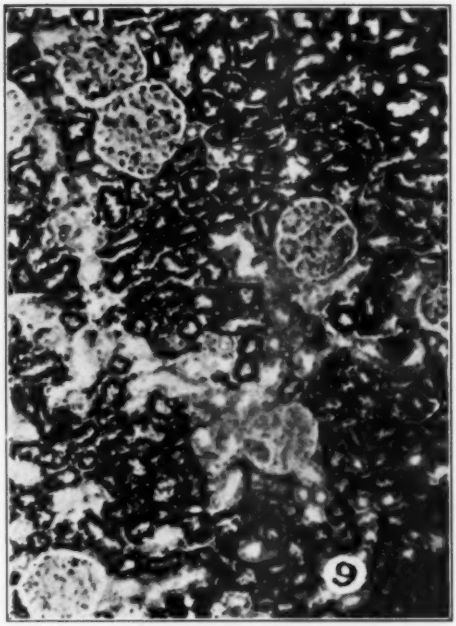

Plate 9.

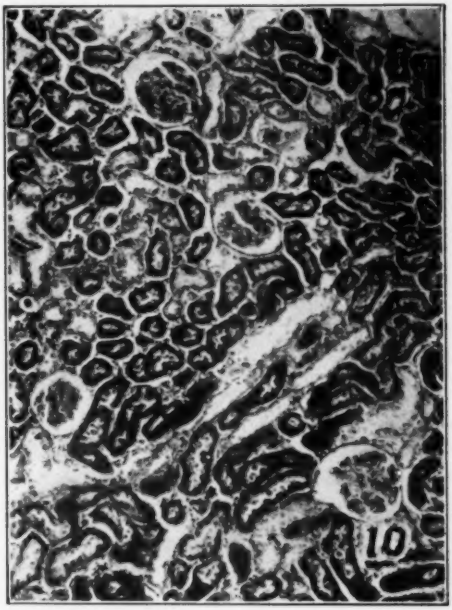

Plate 10.

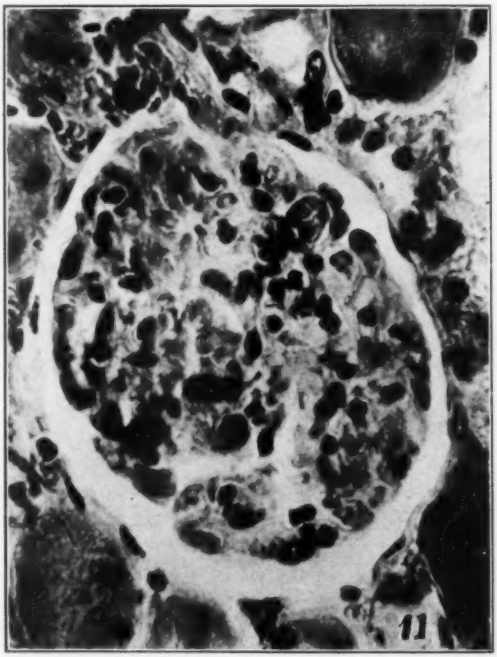

Plate 11.

PRELIMINARY NOTE ON THE TRANSMISSION OF THE EYE WORM OF AUSTRALIAN POULTRY

by

J. W. FIELDING

(Australian Institute of Tropical Medicine, Townsville).

(Submitted for publication 10th November, 1926.)

The occurrence of eye worms of poultry in Australia was first drawn attention to by H. Fern, Poultry Expert of the Queensland Government prior to 1907. Tryon (1907-8) drew attention to Fern's findings. Dodd (1909) recorded its occurrence in the Rockhampton district, and forwarded specimens to Sweet (1910), who described it as *Oxyspirura parvovum*. Tryon (1926) questions the validity of Sweet's new species, and states that it is not generally accepted. But until a comparison of a long series of this species and Cobbold's *Oxyspirura mansoni* has been made it is advisable to allow the Australian species to stand as at present. There is obviously a great variation in the size of worms, which is reflected in the measurements of the various organs, showing a corresponding increase or decrease as they depart from the average. Johnston (1910) recorded the occurrence of *O. mansoni* in New South Wales. Breinl (1913) and Nicoll (1914) both drew attention to the occurrence of *O. parvovum* in North Queensland, and the occurrence of eye worms in the muscovy duck, *Cairina moschata,* in North Queensland is here recorded. Dr. G. M. Heydon informs me of the occurrence of eye worms, which were identified as *Oxyspirura mansoni,* in fowls at Rabaul, in the mandated territory of New Guinea. According to Ransom, Dodd, and various other authors following them, this parasite occurs only on the sea coast; but this is not correct, at any rate so far as Queensland is concerned, as the present writer has found them in inland centres, viz., Charters Towers, Hughenden, etc. Ozoux (1910) recorded the occurrence of *O. mansoni* in the mountainous districts, as well as the coast of the Isle of Reunion. There have been various suggestions as to the mode of infection with eye worms. Emmerez and Megnin (1901) state that the eggs are laid outside the eye of the fowl, but do not state how, or give any evidence in support of their theory that development takes place in water. Ransom (1904) apparently performed a few experiments, with negative results. Ozoux (1910) appears to have carried out some work on the same lines as Ransom, but does not give details of his experiments. He states the worms die after 4–5 hours at 37°–38°, and disintegration

takes place after 48 hours in the dark. He further states that the experiments tabulated allow him to consider that the eggs are hatched outside the body, probably on the soil, and are taken up by the host into the digestive tract, from which they reascend to the eye. He considers that the infection is not insect-borne. Emmerez (1918) states that the females lay their eggs in the eye, or are carried through the tear duct to the mouth, and thence into the intestine, where their bodies dissociate, thereby liberating the eggs, which are expelled in the stool, and hatch on the ground, the larvae living in the wet ground, eventually getting into the eyes of the birds when they take their dust bath.

In our observations, which have been carried out over a number of years, worms have been recovered alive in the mouth, oesophagus, and crop; and the eggs in the mouth, oesophagus, crop, along the alimentary tract, and in the droppings. These eggs hatched after varying periods from 24 hours upwards. The adults have been kept alive in various solutions for a number of days under laboratory conditions. Drinking water has been examined systematically after being centrifugalized, with negative results. The blood was examined on numerous occasions, but found to be negative in all cases for larvae. The eye fluid was also examined for the presence of eggs or larvae, with negative results. The pus-like material which is found in the eyes of infected birds was examined, and it was found that there are two kinds of matter: Firstly, a hard mass of old-standing material, and, secondly, a fresher and softer material, both of which, in our opinion, are the result of decomposition of the worms themselves. When a heavy infection takes place it is usually associated with catarrhal conditions, which block the passages; in consequence there is no outlet for the worms, which are in great numbers, and they eventually die and decompose. In the hard or old-standing material no eggs were seen in numerous examinations, but in the fresher and softer pus eggs were frequently recovered.

Examinations of the shady portions of the infected yard, where the fowls are in the habit of gathering together, revealed the fact that the ground was invariably riddled with filariform larvae, and showed a tremendous increase in the positive findings of nematode larvae over other portions of the yard. Concentrating on the possibility of the parasites being mechanically transmitted by flies, a number of bottle fly traps, containing various solutions, were set. These solutions were taken to the laboratory for examination. They were centrifugalized, and the sediment examined with a $\frac{1}{6}$" objective. This was done daily for six weeks, but only a single nematode larva was seen, which was obviously not the worm under consideration.

An examination of a number of chickens and young ducks was carried out, and showed the presence of eye worms, in the case of the chickens after the age of 10 days had been attained, and in the case of ducks 14 to 21 days. This suggested placing a number of older birds, which had been given a course of

treatment and kept under observation for three weeks and found negative,[1] in a special pen in an infected yard, with no precautions. After six weeks they were examined, and 83·3% were found to be heavily infected. In a somewhat similar experiment, but in this allowing the birds to mix with the other stock, and examining the eyes every three days, three birds were started; one was found to be infected after the expiration of 12 days, one after 15 days, and the remaining one became infected after 35 days.

The preceding observations tending to show, as they did, that the parasites were fairly well developed when introduced into the fowl, suggested a new line of attack, viz., that the infection was insect-borne, and an attack was made on the insect population of poultry yards.

The first to receive attention was the fowl tick, *Argas persicus*, but a long series of dissections and examinations proved negative; fowl lice, *Menopon* sp., gave the same results; the flies, *Sarcophaga* sp., *Musca domestica*, *Stomoxys calcitrans*, and a small unidentified fly also proved negative, as also did a large series of examinations of mites, *Dermanyssus gallinae*, and a number of crickets, *Nemobius* sp. Attention was then directed to the cockroaches, *Periplaneta australasiae* Fab. and *P. americana* Linn., and a number of adults and young forms gave negative results. But on concentrating on another species of roach, *Leucophaea surinamensis* Linn., we were immediately struck by the presence in the body cavity, in the nymphal and adult stages, of filariform worms, which appeared macroscopically and microscopically to be immature stages of the eye worm.

There were also usually associated with these worms numerous capsules containing larvae. These capsules varied in size as the development of the larvae proceeded in them, sometimes attaining 3 by 1·5 mm., of four measured accurately the measurements being 2·7 by 1·3 mm., 2·3 by 1·4 mm., 2·2 by 1·3 mm., and 1·8 by 1·1 mm.

On examining a long series of cockroaches, dividing them into three batches, the percentage of infection was as follows:

(*a*) Young and very young forms, no infection present.
(*b*) Intermediate forms showed an infection rate of 38%.
(*c*) Adult forms, an infection rate of 93%.

This gave a percentage infection rate of the total examined of 56%. The number of worms obtained from each cockroach averaged 21. In numerous individuals the total number recovered was small, viz., 2 and 3, but in one case a total of 108 worms and capsules were obtained, and in others similar high numbers were observed.

[1]This treatment was given irrespective of whether the birds were infected or uninfected before proceeding with an experiment, on account of the fact that almost all yards were more or less infected.

An examination of a number of cockroaches was carried out, with a view to obtaining information regarding the length of time the worms could live after the death of the insect. Some of the roaches were just on the point of dying; others had been dead for varying periods up to a maximum time of 64 hours. The examination showed that the activity of the worms was just as great in the roaches which had been dead the maximum time, even though in these cases decomposition was in an advanced stage. Further work is proceeding on these lines. Arrangements were then made to carry out experiments to prove whether the worms so obtained were eye worms. Young birds were obtained from an uninfected yard, and were kept under observation for some days prior to experiment. The young ducks were hatched in the ordinary way, but the young chicks were incubator bred.

Experiment 1. One duckling, about a week old, was fed with four cockroaches in the nymphal stage; it was examined 17 hours later, and found to be infected with eye worms.

Experiment 2. Four ducklings from the same batch as (*a*) were fed with three cockroaches, each in the nymphal stage, by pushing them whole down the throat, and when examined 17 hours later were all found to be infected.

Experiment 3. One duckling was given seven worms, extracted from infected roaches, the worms being placed on the tongue with a camel hair brush. On examination 17 hours later it was found to have worms in both eyes. Control ducks were kept, and remained negative during the time the birds were under observation.

Experiment 4. One duck, 17 days old, was given cockroach entrails with capsules attached (one worm was just emerging), the whole being placed in the mouth at 12.46 p.m.; when examined at 12.58 p.m., 12 minutes after introduction, worms were found in both eyes. Control negative.

Experiment 5. One duck, 17 days old, was given 5 worms, taken from cockroaches; 13 minutes after introduction into the mouth they were found in the eyes. Control negative.

Experiment 6. One duck, 17 days old, was given 10 cockroaches which had been etherized. It was examined 10 and 20 minutes afterwards. There being no sign of eye worms, a further 5 roaches were introduced, and the examination carried out at five-minute intervals. At the expiration of 55 minutes worms were found in both eyes. No control kept.

Experiment 7. Eight young chickens, 9 days old, were each given 4 whole cockroaches in the nymphal stage; a few extra roaches were then scattered in the box, and were picked up by the chickens. Two and a half to three hours afterwards the chicks were all infected. Controls negative.

Experiment 8. Three young chicks, 10 days old, were given 1 adult cockroach each, between 3.35 and 3.40 p.m. They were then constantly examined for the presence of worms in the eyes; this occurred at 3.58, i.e., 18 to 23 minutes

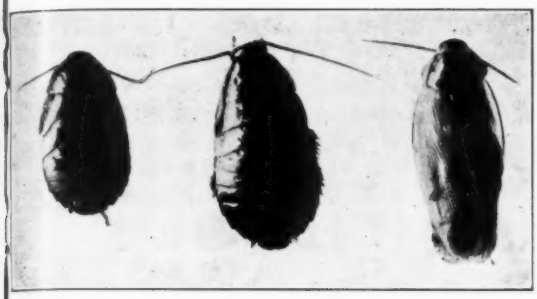

Fig. 1. × 1·7.

Fig. 3. × 3·8.

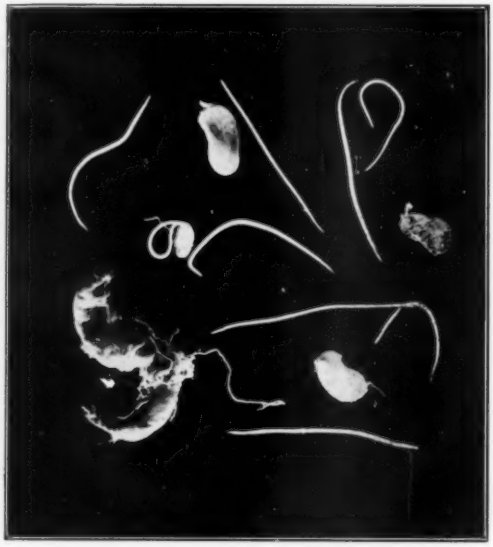

Fig. 2. × 3·8.

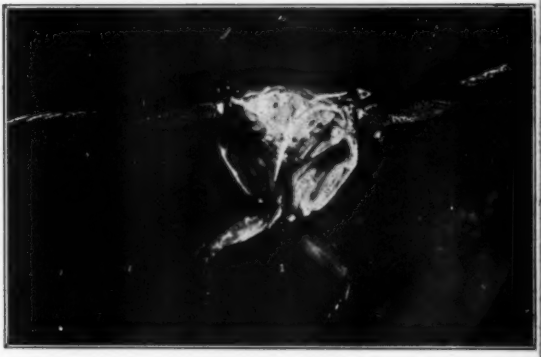

Fig. 4. × 3·8.

F. H. Taylor, Photo.

Fig. 1. Showing adult male and miniature females of cockroach, *Leucophaea surinamensis*, Linn.

Fig. 2. Showing worms, cysts, and portion of gut with cysts attached, taken from *Leucophaea surinamensis*.

Fig. 3. Showing head of *Leucophaea surinamensis* with worm protruding.

Fig. 4. Showing third pair of legs and portion of body of *Leucophaea surinamensis*, with two worms protruding.

after the introduction of the cockroaches. Care was exercised in introducing the insects to the birds, so as to ensure that the former should not be ruptured during the process. Control negative.

Experiment 9. One chick, 11 days old, was given 3 adult cockroaches, care being taken not to rupture them. The chicks were then kept under constant observation, and in exactly 17 minutes after the feeding worms were noted in the eyes. No control was kept.

Experiment 10. One adult muscovy duck, which was examined thoroughly for the presence of eyeworms, and found to be negative, was then given 8 cockroaches, taking care not to rupture them. The mouth and eyes were then kept under constant observation, and exactly 8 minutes after the introduction worms were seen on the roof of the mouth, and on the tongue, 9 worms in these positions being counted. After this the eyes were watched constantly, but worms did not appear until 31 minutes from the start, i.e., 23 minutes after their presence in the mouth. It was interesting to note that worms were seen on five occasions passing into the eyes from the naso-lachrymal duct. No control was kept.

It was thought advisable, in view of the theory that the cockroaches do not go beyond the crop before the worms are liberated, and considering the short space of time occupied between the introduction of the cockroaches and the appearance of the worms in the eyes of the bird, to note the fate and position of the cockroaches in an allotted time. For this purpose four young chicks were killed at varying intervals, viz., 5, 10, 15, and 30 minutes, after the introduction of the insects.

Experiment 11. A chick, 16 days old, was given 1 adult cockroach, and after an interval of 5 minutes was killed, the insect being found at the entrance of the crop, on the top of the contained foodstuff. On examination of the roach there was no apparent change noted. No free worms were observed.

Experiment 12. A chick, 16 days old, was given 1 adult cockroach, and after an interval of 10 minutes was killed. Except that the cockroach was in the middle of the foodstuff, no difference was observed. No free worms were seen.

Experiment 13. A chick, 16 days old, was given 1 adult cockroach, and after 15 minutes was killed, the roach being found at the entrance to the crop, on top of the foodstuff. The skin of the insect was observed to be in a fairly soft condition, and a worm was seen just emerging from the soft part of the under-surface of the body, between the third and fourth leg. No free worms were seen before dissection.

Experiment 14. A chick, 16 days old, was given 2 adult cockroaches, and after 30 minutes the chick was killed; the roaches were on top of the foodstuff, at the entrance of the crop, and were found to be very soft, and easily broken

at the slightest touch with the dissecting needles. No worms were noted until the cockroaches were dissected, when a fair number were found.

It would appear, although only one escaping or escaped worm was seen in the preceding series of experiments, that the cockroaches do not pass beyond the crop within the limits of the time necessary for an infection to take place, and that the digestive juices are responsible for the softening of the tissues, and probably the increased temperature has a stimulating effect on the worms, which break through the softer portions of the body. This probably applies only in cases where the roaches were swallowed intact, which would occur only in a small percentage of cases, as it is very noticeable that the birds peck at the insect, and by so doing rupture most of them, thus making an easy exit for the escape of the worms.

From the foregoing experiments there appears to be indisputable evidence that the cockroach, *Leucophaea surinamensis* Linn., is a responsible agent for the transmission of the eye worms of fowls and ducks. The cockroaches apparently ingest the young larvae from the droppings and from the ground. Having attained their objective, the larvae then pass through the wall of the alimentary tract, on the outside of which they encapsulate. In the capsules development takes place, and on attaining the stage of infectivity, they leave the capsules, and wander about in the body cavities. They have been recovered from both the thoracic and abdominal cavities, as well as the legs. The infected cockroaches are in their turn taken up by the poultry, and in our opinion do not pass further than the crop before the worms which have attained the infective stage escape from the intermediate host; the parasites then pass up the oesophagus to the mouth, and eventually through the naso-lachrymal duct to their destination. There now remains to examine in detail two stages in the life history of this parasite. It is anticipated that this will take come considerable time to complete, as in (*a*) it will be necessary to feed one or two fowls with large numbers of infected cockroaches, so that heavily infected birds may be available for ascertaining the life history of the worm in the fowl, particularly the number of ecdyses and changes that occur in the worms before they attain maturity and reproduction begins, and (*b*) to obtain information regarding the time taken by the larvae to reach the infective stage after entering the cockroach.

It would appear that there are five distinct stages in the development of this parasite, quite apart from any changes which occur during each stage:

1. Embryonic stage, portion of which is spent in the parent worm, portion in the alimentary tract of the bird, and portion on the ground.

2. Larval stage, portion of which is spent in the ground, and portion in the alimentary tract of the cockroach.

3. Cystic stage, in capsules on the outside of the alimentary tract.

4. Free or infective stage, in body cavities, legs, etc.

5. Pre-adult and adult stage, in the eyes of the birds, where development is completed.

Treatment.

The first necessity in getting rid of eye worms, now that there is a known intermediate host, is obviously a concentrated attack on all cockroaches, and it would appear that the best method is trapping, since poisoning and fumigation are too dangerous to poultry. The yard should be cleaned of all droppings, and sprayed well with a good disinfectant frequently. The poultry should then be treated, preferably by placing a few drops of turpentine in the eyes, and allowing it to remain for half an hour, then irrigating with lukewarm water or boracic water, and removing worms from the eyes with a small camel hair brush. Although this treatment is undoubtedly severe, it has the advantage of being quick in action. Weak Condy's fluid is also recommended, but is much slower, and requires some days to obtain the required results. Some poultry-keepers use kerosene, and find that it gives good results.

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ON THE ACCELERATION OF ENZYMATIC SYNTHESIS OF PROTEINS BY LIPOIDAL EMULSIONS

by

HEDLEY R. MARSTON

(From the Darling Laboratories of Physiology and Biochemistry, University of Adelaide).

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The fact that the proteolytic enzymes are able to catalytically resynthesize protein from its hydrolytic products was pointed out concurrently by T. Brailsford Robertson (3, 4) and A. E. Taylor (6). To demonstrate the reversal of the hydrolytic activity of the enzyme Robertson utilized pepsin and the peptic digestion products of casein, and Taylor, a tryptase from the liver of the Californian clam, *Schizothoerus nutalli*, and the tryptic digestion products of the simple protamine salmin. Robertson, in collaboration with Gay (1), was able to prove that the product of the reaction was antigenic, and identical with casein or one of its early cleavage-products. He also showed that the synthetic reaction velocity was increased by heating above the optimal temperature for the hydrolytic activity of the enzyme, and that a high concentration of enzyme favoured synthesis, the former fact leading him to consider that the synthesizing enzyme was a tautomeric anhydride of the hydrolyzing enzyme.

Wasteneys and Borsook (7) have recently confirmed the earlier findings of Robertson, and extended our knowledge by demonstrating the importance of the hydrogen ion concentration in the reversal. They showed without doubt that the synthesized material was of the same order of complexity as the native protein.

The remarkable rapidity with which the living cell is able to repair itself after inanition, and to synthesize specific products during proliferation, clearly points to the occurrence within the cell of a physico-chemical mechanism which favours synthetic activity. This has lead to the hypothesis that such a site exists in the mitochondria (2), the water poor phases existing at the surface of the colloidal lipoids in these bodies instituting a favourable set of conditions for the enzyme to exert its synthetic activity.

More recently Robertson (5), in extending this hypothesis, pointed out that the lipoids would tend to orient the molecules of amino acids at the surfaces, and bring the reactive amino and carboxyl groups into close proximity, and to point them towards the aqueous phase, whereas the lyotrope hydrocarbon chains

would be buried in the lipoid phase. The effect of thus concentrating and bringing into close proximity the reactive groups would be to favour synthesis, even if the catalyst were not in high concentration at the synthetic site, but as we are led to believe from other evidence (2) that the proteolytic enzyme is concentrated in the mitochondria, wherein the main lipoid phases of the cytoplasm of the cell occur, it becomes clear that practically ideal conditions for the building up of proteins exist in these bodies.

Before the latter publication (5) appeared the author had observed in several experiments that the rate and completeness of resynthesis of protein complexes from the hydrolytic products of casein by the action of pepsin were greatly influenced if emulsions of lecithin and cholesterol were added to the reaction mixture, the lipoid emulsions tending to make the final production of protein-like bodies more complete, and to increase the reaction velocity of the synthesis.

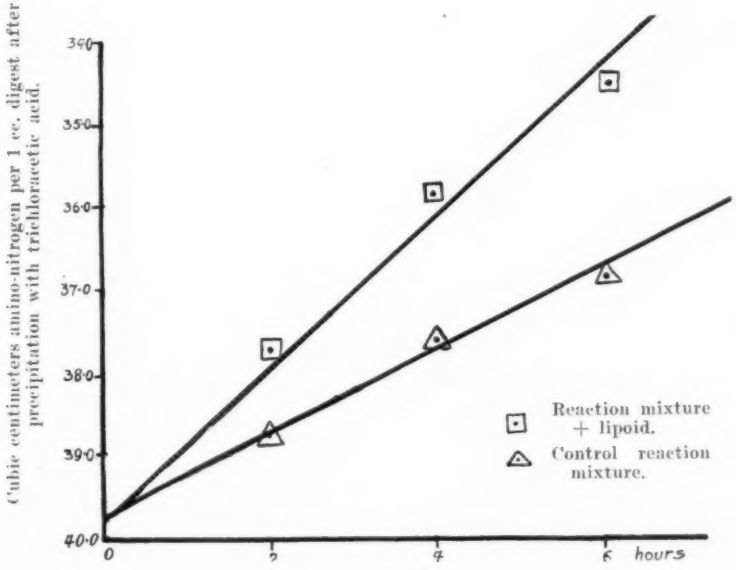
That lipoid emulsions also increase the reaction velocity of the synthetic activity of trypsin is evident from the following experiment:

A substrate was prepared by digesting a 5% solution of egg albumin in a ·1 mol. phosphate-citrate buffer with trypsin, separated from pigs' pancreas, for several days, until a complete absence of protein was indicated by the failure to produce a precipitate with trichloracetic acid. The digest was boiled for five minutes to inactivate the enzyme, and was then concentrated to one-fifth its bulk in a serum drier at 40°C. The solution was preservatized throughout by the addition of toluol. It was adjusted to P_H 5·8, which Wasteneys showed was approximately the optimum reaction for the tryptic synthesis, by first establishing its titration curve against ·1 normal citric acid by means of the gold-quinhydrone electrode, the potentials being estimated with a high precision potentiometer equipment.

The trypsin employed was separated from acetone-exhausted pig's pancreas by extraction with water, and after filtering the concentrated extract it was adjusted to P_H 7·5, and digested at 35°C. for 8 hours, and then dialyzed for 8 hours against a stream of distilled water in a close celloidin sack, into which was introduced a fairly high-speed mechanical stirrer. The enzyme was then either precipitated by the addition of 10 volumes of absolute alcohol or frozen in aqueous solution at —5°C., and stored until wanted at this temperature.

The lipoid emulsion was prepared from egg yolk by the following method: The yolks of fresh eggs were separated from the whites as completely as possible, and stirred into twice their bulk of anhydrous sodium sulphate. After drying, the mixture was powdered and extracted in a continuous extraction apparatus with ether. The ether was then removed from the extracted fats, and the residue was taken up in alcohol, so as to obtain a solution of about 30% fat. This solution was poured into water whilst stirring with a high-speed electric stirrer. By this procedure a very stable emulsion may be prepared, containing upwards of 20%

fat in a condition which is remarkably stable to changes in hydrogen ion concentration, and change in concentration of neutral salts. The alcohol is separated by distilling off under reduced pressure at 40°C. Pure lecithin emulsions were discarded in favour of those of mixed egg lipoids, owing to the extreme lability of the former.



Two 50 cc. portions of the substrate were adjusted to a reaction of P_H 5·8 by the addition of the required amount of citric acid, as calculated from the titration curve of 10 cc. of the same substrate previously determined. These were then introduced into long-necked, stoppered flasks, which were immersed under the surface of the water in a thermostat at 40°C. (±·01°C.). To one, 5 cc. of water was added; to the other 5 cc. of the concentrated lipoid emulsion, and after the contents of the flasks had reached that of the bath, 1 cc. of 20% trypsin, which had previously been brought to the temperature of the bath, was added to each flask.

At the times indicated on the protocol 1 cc. was withdrawn from each mixture, and run into 10 cc. of 2% trichloracetic acid, and the whole made up to 20 cc., filtered, and the amino-acid content estimated at once in 2 cc. of the filtrate by means of a micro Van Slyke apparatus. The analyses were done in duplicate, and where the second differed more than 1% from the first estimate a third analysis was performed.

The results show clearly the increase in velocity of the synthetic action due to the colloidal lipoid phase. The synthetic reaction is practically linear over the range studied, but this represents but a small portion of the complete reaction. From the linear slopes the reaction velocity is almost doubled under the conditions of this experiment. Precipitated protein was noted in the reaction mixture containing lipoid after about 90 minutes, and appeared in about 150 minutes in the control digest.

SUMMARY.

Colloidal emulsions of egg fat greatly increase the reaction velocity of the resynthesis of protein from its digestion products by trypsin.

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